

Applicants: Dean Engelhardt et al.  
Serial No.: 08/479,997  
Filed: June 7, 1995  
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not all of the application numbers cited in the specification were updated and because on page 65 a question mark appears in the margin. Applicants have hereinabove amended the specification to delete the question mark on page 65, and to correct several typographical errors in the specification. None of these corrections raise an issue of new matter.

With respect to the Examiner's assertion that the application numbers cited in the specification were not updated, applicants direct the Examiner's attention to the Second Preliminary Amendment submitted September 18, 1995 in the present application. On pages 2-3 of this amendment, applicants updated the cross-reference to related applications and the citation on page 2 of the application to Serial No. 255,223. If there are other citations to applications that the Examiner believes require updating, applicants request that they be advised accordingly.

The Examiner in the Office Action also objected to the specification and rejected claims 207-224 and 227-262 under 35 U.S.C. §112, first paragraph. The Examiner alleges that the disclosure does not provide support for the invention presently claimed. Specifically, the Examiner maintains that there is no support or explicit description of the claimed products wherein Sig is bound to the phosphate moiety. The Examiner states that an explicit description of the phosphate-Sig reactions would have been expected in order for the skilled artisan to have reasonably concluded that the original disclosure evidenced "possession" of the currently claimed invention. Since, in the Examiner's opinion, there is no support for claims wherein the Sig is attached to the phosphate moiety, he has deemed these claims as "new matter".

The Examiner has also objected to the specification and rejected claims 204-224 and 227-262 under 35 U.S.C. §112, first paragraph, alleging that the specification fails to adequately teach how to make and/or use the invention, i.e. the specification fails to provide an enabling disclosure. In this regard, the Examiner maintains that the specification, particularly Examples I-VII, provides sufficient detailed description to enable the construction of base-labeled nucleotides wherein the Sig moiety is linked to the base. The Examiner, however, maintains that there is no analogous disclosure for the attachment of the Sig moiety to the phosphate moiety of the nucleotides.

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Applicants respectfully disagree with the Examiner's positions and contend that the original disclosure is enabling and provides adequate support for the claims in which Sig is attached to the phosphate moiety. Specifically, applicants direct the Examiner's attention to Example V. This example, contrary to the Examiner's assertions, describes the labeling of the phosphate moiety with Sig. The example refers to an article by Halloran and Parker, *J. Immunol.*, 96: 373 (1966), in which a mechanism is described for coupling nucleotides to proteins. The described mechanism involves the formation of nitrogen-phosphorous bonds with the protein's amino groups in the presence of carbodiimides. By utilizing the procedure of Halloran and Parker, Example V sets forth an exemplary labeling of the phosphate moiety of oligonucleotides with biotin and polybiotinylated poly-L-lysine in the presence of carbodiimides. From Example V, it is evident that at least one means of coupling nucleotides and oligonucleotides to labels through the phosphate moiety was available to those skilled in the art at the time of the invention.

Applicants also point out that at the time of the invention mechanisms were known in the prior art for the binding of polynucleotides to cellulose through phosphodiester linkages at the 5'-terminals or 3'-terminals of the polynucleotides chains. In view of the prior art (including the Halloran and Parker article), Example V in the specification, and the other teachings set forth in the disclosure, applicants maintain that the Examiner's contention that there is no explicit description of the claimed nucleotides and polynucleotides wherein the label is bound to the phosphate moiety is incorrect. The application as originally filed provides adequate support and an enabling disclosure for labeling the phosphate moieties of nucleotides and polynucleotides with Sig.

Furthermore, applicants wish to point out that the disclosure of the present application has been deemed by the U.S. Patent Office as adequate support for claims wherein Sig is attached to the phosphate moiety of polynucleotides. U.S. Patent No. 5, 260,433, which issued November 9, 1993, derives from the same parent application as that of the present application and hence has the same disclosure. In this patent, claims were allowed wherein Sig is attached to the phosphate moiety - see for example claim 1, subpart (ii).

Accordingly, in light of the above arguments, applicants respectfully request that the Examiner reconsider and withdraw the objections and rejections under 35 U.S.C. § 112, first paragraph.

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Claims 204-206, 217, 234-240, 251, 259, and 261 were also rejected under 35 U.S.C. §102(b) as anticipated by Mackey et al. The Examiner maintains that Mackey et al. teach a radioactive signal moiety attached to the phosphorous of a nucleotide and an oligonucleotide.

Applicants respectfully disagree with the Examiner's position and maintain that nowhere in the Mackey et al. reference is a Sig moiety attached to a phosphorous of a nucleotide or oligonucleotide. Rather, the Mackey et al. reference discloses the use of radioactive isotopes of phosphorous in building the nucleotide or oligonucleotide. In other words, the oligonucleotides disclosed in Mackey et al. have one or more of their phosphorous atoms substituted with radioactive  $^{32}\text{P}$  isotopes. This is not a chemical modification or a chemical labeling of the phosphate moiety of a oligonucleotide but rather, merely a substitution of a radioactive isotope of phosphorous for the non-radioactive phosphorous typically present in the oligonucleotide. Nowhere in the reference is it disclosed that a Sig moiety is covalently attached to the phosphate moiety of the oligonucleotide as set forth in the claims. In fact, no moiety is attached to the phosphate moiety.

The claims of the present invention require that a Sig moiety, which is a detectable moiety, be attached to the phosphate moiety of the nucleotide or oligonucleotide. In the case of radioactive labels, this typically requires that the radioactive atom be carried by a chelator or label which in turn is covalently bound to the phosphate moiety of the nucleotide or oligonucleotide, e.g. a sugar label with a radioactive hydrogen isotope bound to the phosphate moiety.

In sum, the Mackey et al. reference fails to disclose or suggest the presently claimed invention. Accordingly, applicants request that the Examiner reconsider and withdraw this rejection.

The Examiner also rejected claims 215, 216, 221-224, 231, 233, 249, 250 and 255-258 under 35 U.S.C. §103 as unpatentable over Gohlke et al (U.S. Patent No. 4,378,458). The Examiner maintains that Gohlke discloses the use of detection assays using labels such as fluorescent compounds, chemiluminescent compounds and enzymes like  $\beta$ -galactosidase and antibodies. The Examiner alleges that the claims differ from Gohlke only by their explicit recital of the attachment being at the phosphate moiety. The Examiner also maintains that

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such attachment at the phosphate moiety was *prima facie* obvious to one skilled in the art at the time of the invention.

Applicant respectfully disagree with the Examiner's position regarding Gohlke et al. Initially, applicants point out that there is an inherent inconsistency in the Examiner rejecting the application under 35 U.S.C. §112 by arguing that the disclosure is not enabling to permit one skilled in the art to practice the invention by labeling the phosphate moiety with Sig and then in the next breath arguing that the invention is obvious to one skilled in the art based on the Gohlke reference. How can the invention not be enabled by the specification in view of the prior art and at the same time be obvious in view of the prior art?

With respect to the Gohlke reference, applicants also maintain that the reference does not teach a general labeling technique for oligo- or polynucleotides. The method disclosed in the Gohlke patent would not be applicable to DNA since DNA would not dissolve in the solvent used in the procedures of the patent. Additionally, the procedures set forth in the Gohlke patent require acetylation of the bases of the polynucleotides because of the acid conditions (see example 2). Such conditions would destroy DNA and a polynucleotide's ability to hybridize. In the examples of the Gohlke patent, nucleotides with uridine are used because of uridine's resistance to acetylation (see example 1).

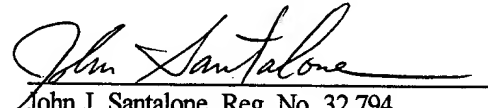
Moreover, in example 3 of the Gohlke patent, the sugar ring is open in order to label the nucleotide. Such a procedure would likewise destroy the ability of polynucleotides labeled in this manner from hybridizing. Additionally, the procedure of the Gohlke patent is not universally applicable to all polynucleotides or nucleotides since HCl is used, which would react with modifiable groups on the bases. In sum, the procedure disclosed in Gohlke is not a method which can be used generally for labeling polynucleotides, and specifically for labeling DNA.

The Examiner rejected claims 207-214, 219, 220, 227-230, 232, 241-248, 253, 254, 260 and 262 under 35 U.S.C. §103 as unpatentable over Gohlke in view of Sodja. The Examiner alleges that Sodja teaches the attachment to the free 3' OH end of RNA an avidin-ferritin label using the lysine groups of the polypeptide cytochrome-c. The Examiner maintains that it would have been *prima facie* obvious to one of ordinary skill in the art at the

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In addition to the fee for the extension of time, \$330 is due for the addition of new claims. Authorization is hereby given to charge this fee to Deposit Account No. 05-1135. If any other fee is deemed necessary, authorization is hereby given to charge the amount of such fee to Deposit Account No. 05-1135.

Respectfully submitted

  
John J. Santalone, Reg. No. 32,794  
Attorney for Applicants

Enzo Diagnostics, Inc.  
c/o Enzo Biochem, Inc.  
527 Madison Avenue, 9th Floor  
New York, New York 10022  
Tel: 212-580-0100